Bone turnover markers predict hip bone loss in elderly European men: results of the European Male Ageing Study (EMAS)

Evelien Gielen, MD [1], Terence O'Neill, MD PhD [2], Stephen Pye, MPhil [2], Judith Adams, MD [3], Kate Ward, PhD [4], Frederick Wu, MD PhD [5], Michaël Laurent, MD [1,6], Frank Claessens, PhD [6], Steven Boonen, MD PhD [1†], Dirk Vanderschueren, MD PhD [7], Sabine Verschueren, PhD [8]

[1]Gerontology and Geriatrics, Department of Clinical and Experimental Medicine, KU Leuven, Belgium & Centre for Metabolic Bone Diseases, UZ Leuven, Belgium; [2]Arthritis Research UK Centre for Epidemiology, Centre for Musculoskeletal Research, Manchester Academic Health Science Centre (MAHSC), The University of Manchester, UK; [3]Manchester Academic Health Science Centre (MAHSC) and Radiology at Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust, UK; [4]Nutrition and Bone Health, Medical Research Council Human Nutrition Research, Cambridge, UK; [5]Andrology Research Unit, Manchester Academic Health Science Centre (MAHSC), The University of Manchester, UK; [6]Laboratory of Molecular Endocrinology, Department of Cellular and Molecular Medicine, KU Leuven, Belgium; [7]Clinical and Experimental Endocrinology, Department of Clinical and Experimental Medicine, KU Leuven, Belgium; [8]Research Group for Musculoskeletal Rehabilitation, Department of Rehabilitation Sciences, KU Leuven, Belgium

Corresponding author: Evelien Gielen, Gerontology and Geriatrics, UZ Leuven, Herestraat 49, B-3000 Leuven, Belgium. E-mail: evelien.gielen@uzleuven.be. Tel +3216340931. Fax +3216342641.

Abstract

Purpose: Prospective studies on the value of bone turnover markers (BTMs) to predict changes in areal BMD (aBMD) in men are few and conflicting. The aim of this study was to determine whether BTMs predict changes in aBMD in middle-aged and elderly European men.

Methods: In 487 men aged 40-79 years from the European Male Ageing Study (EMAS), BTMs were assessed at baseline and DXA at the lumbar spine (LS), femoral neck (FN) and total hip (TH) was performed at baseline and after a mean follow-up of 4.3 years.

Results: The mean aBMD decreased by 0.32%/year at FN and 0.22%/year at TH, and increased by 0.32%/year at LS. Higher baseline levels of β C-terminal cross-linked telopeptide (β-CTX) and N-terminal propeptide of type I procollagen (PINP) were significantly associated with higher loss of hip aBMD in the whole cohort and men aged 60-79 years. These associations remained significant after adjustment for age, centre and BMI. Men aged 60-79 years with β-CTX in the upper quintile were more likely of being in the upper quintile of annual % aBMD loss at FN (OR=4.27;95%CI=2.09-8.73) and TH (OR=3.73;95%CI=1.84-7.57). The positive predictive value (PPV) was 46% at both hip sites.

Conclusion: Older men with high bone turnover have a higher risk of accelerated hip bone loss, but the PPV is low. BTMs are therefore unlikely to be of clinical utility in predicting accelerated hip bone loss in individual subjects.

Mini Abstract

The aim of this study was to determine whether bone turnover markers (BTMs) predict changes in aBMD in middle-aged and elderly European men. Older men with high bone turnover are at higher risk of accelerated hip bone loss, but the clinical utility of BTMs in individuals is limited.

Key words

bone turnover markers; bone mineral density; prospective study; men; osteoporosis

Introduction

Osteoporosis in men is an important clinical and public health problem. From age 50 years on, it is estimated that 1 in 3 of all fragility fractures occur in men. Further with the demographic shift towards an ageing population, the number of men with fragility fractures is set to increase substantially [1,2]. Areal bone mineral density (aBMD) is a major determinant of fragility fracture risk and declines with age in both genders, but earlier and to a greater extent in older women, presumably because men better maintain bioavailable estrogen levels than women and do not experience an accelerated phase of bone loss as women do at the menopause [3].

Several large population-based studies in postmenopausal women have shown that markers of bone turnover modestly predict bone loss and fracture risk [4,5]. In contrast to women, less is known about the potential influence of bone turnover on changes in aBMD in middle-aged and elderly men. There are indeed relatively few prospective data in men and most associations have been examined in or include an elderly population [6-11], with few data in younger men only (<60 years) [12]. Furthermore, these studies have provided inconsistent results [13]. At the total hip, bone turnover was associated with bone loss over 5 to 7.5 years in two large cohorts (MrOS and MINOS) [6,7], though at the femoral neck it was not associated with bone loss in the majority of studies [6,8-11,14]. At the lumbar spine, baseline bone turnover was positively associated with change in aBMD over a 3 year follow-up [10], but not over a 4 to 10 year follow-up [6,8,11].

Using cross-sectional data from the European Male Ageing Study (EMAS), we previously showed a negative association between markers of bone turnover and aBMD, which remained significant after adjusting for lifestyle and endocrine factors, including physical activity, smoking, alcohol intake, estradiol (E_2), testosterone (T), sex hormone-binding globulin (SHBG), parathyroid hormone (PTH) and insulin-like growth factor-1 (IGF1) [15]. There are

no prospective data in men which have examined whether any observed relationship between bone turnover markers and loss of aBMD is independent of lifestyle and hormones.

The aim of this study was to determine i) the magnitude of change in aBMD in an age-stratified sample of middle-aged and elderly European men; and ii) whether baseline levels of bone turnover independently predict changes in aBMD.

Materials and Methods

Subjects

Men aged 40-79 years were recruited from population registers in Manchester (UK) and Leuven (Belgium) for participation in EMAS, a prospective study of male ageing. Details concerning the study design and recruitment have been described previously [16,17]. Briefly, in 2003-2005, community-dwelling men were invited to attend by a letter of invitation which included a postal questionnaire. Those who agreed to take part were invited to attend a local clinic for assessments. In 2007-2009, participants were invited to take part in a repeat survey. Ethical approval for the study was obtained in accordance with local institutional requirements in each centre. All subjects provided written informed consent.

Study questionnaires and clinical assessments at baseline and follow-up

At baseline, subjects completed a postal questionnaire which included questions about smoking (ever, never, don't remember) and alcohol consumption in the previous month (every day/5-6 days per week/3-4 days per week/1-2 days per week/less than once a week/not at all). Subsequently, participants attended a research clinic to complete an interviewer-assisted questionnaire and undergo a clinical assessment. The interviewer-assisted questionnaire included the Physical Activity Scale for the Elderly (PASE), which combines information on leisure, household and occupational activity [18]. The questionnaire also

included queries about current prescription and non-prescription drugs. Height and weight were measured in a standardized fashion; height to the nearest 1 mm using a stadiometer (Leicester Height Measure, SECA UK Ltd) and body weight to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA UK Ltd). Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in square metres (m^2).

Biological measurements

A single fasting morning (before 10.00 h) venous blood sample was obtained from all subjects at baseline. Serum was separated immediately after phlebotomy and stored at -80°C until assay at the end of the baseline study.

Methods of measurement of bone turnover markers and hormones have been described in detail previously [19,20]. To assess bone resorption, serum β C-terminal cross-linked telopeptide (β-CTX) was measured on the Elecsys 2010 automated analyser (Roche Diagnostics GmbH) using the β-Crosslaps/serum reagents [21]. This assay is specific for cross-linked β-isomerised type I collagen C-telopeptide fragments and uses two MABs, each recognizing the Glu-Lys-Ala-His-βAsp-Gly-Gly-Arg peptide (Crosslaps antigen). The detection limit was 10 pg/ml and the intra-assay coefficient of variation (CV) <5.0%. To asses bone formation, measurements were performed on the Elecsys 2010 with a two-site assay using MABs raised against intact human N-terminal propeptide of type I procollagen (PINP) purified from human amniotic fluid. This assay detects both intact mono- and trimeric forms (total PINP) [22]. The lower detection limit was <5 ng/ml and the intra-assay CV <3.0%.

Measurement of T and E_2 were carried out by gas chromatography-mass spectrometry as described by Labrie et al [23,24]. The lower limit of quantitation was 0.17 nmol/l for T and 7.34 pmol/l for E_2. The CV within runs was 2.9% for T and 3.5% for E_2. SHBG was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche

Diagnostics, Mannheim, Germany) as described previously [25] and within-assay CV was 1.7%. The free and bioavailable (non-SHBG-bound) T and E_2 levels were derived from total hormone, SHBG and albumin concentrations using mass action equations and association constants as described by Vermeulen et al and Van Pottelbergh et al [26,27]. Serum was assayed for IGF1 using chemiluminescence immunoassay. The detection limit was 20 ng/ml and the within-assay CV 7.4%. Measurement of PTH was carried out by chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH assay, Quest Diagnostics, Madison, NJ, USA). The detection limit was 1.6 pg/ml and within-assay CV 6.0%.

Dual-energy X-ray absorptiometry

At baseline, subjects had dual-energy X-ray absorptiometry (DXA) scans performed on QDR 4500A Discovery scanners (Hologic Inc, Bedford, MA, USA), with measurements of aBMD at the lumbar spine (L1-4), femoral neck and total hip. After a mean follow-up of 4.3 (range 2.7-6.3) years, repeat DXA scans were performed using the same scanners as used at baseline. All scans and analyses were performed by trained and certified DXA technicians. The Hologic Spine Phantom was scanned daily to monitor the device performance and long-term stability. The precision errors of these measurements in Leuven were 0.57%, 1.28% and 0.56% at the lumbar spine, femoral neck and total hip respectively (n=20). In Manchester, these precision errors were 0.97%, 2.04% and 0.97% respectively (n=31). Devices in Leuven and Manchester were cross-calibrated with the European Spine Phantom [28].

Statistical analysis

Descriptive statistics were used to summarize subject characteristics, including age, weight, height, physical activity (PASE), smoking, alcohol consumption, biochemical

markers of bone turnover (β-CTX and PINP), sex hormones (total T, free T, bioavailable T, total E_2, free E_2, bioavailable E_2, SHBG), PTH and IGF1.

Baseline and follow-up aBMD at the lumbar spine, femoral neck and total hip as well as absolute change and annual percentage (%) change in aBMD were calculated in men aged 40-79 years overall as well as in four 10-year age bands (40-49, 50-59, 60-69 and 70-79 years).

The association between bone turnover markers at baseline (categorized in quintiles) and the annual % change in aBMD was assessed visually using bar plots. Linear regression was used to determine the strength of the association between baseline β-CTX and PINP (independent variables in separate models) and annual % change in aBMD (dependent variable), with the results expressed as β coefficients and 95% confidence intervals (CI). To determine whether bone turnover markers were also associated with bone loss in middle-aged men, analyses were not only performed in the whole cohort, but also in middle-aged and elderly men separately, with the mean age of our cohort as the cut-off in order to have an equal number of men in each group. Annual % change in aBMD was calculated as ([follow-up value – baseline value] / baseline value) * 100 / time between DXA scans, therefore taking into account baseline aBMD. For ease of interpretation and comparison we standardized the bone turnover markers into z-scores. Adjustments were made for age, centre and baseline BMI, and also for PASE, smoking, alcohol consumption and serum hormone concentrations (total T, total E_2, SHBG, PTH, IGF1).

Logistic regression was then used to determine whether baseline levels of bone turnover markers predict the risk to be in a different quintile of aBMD at follow-up as compared to baseline ('deviation from tracking'). Logistic regression was also used to determine the association between the highest change in aBMD and high *vs* lower bone turnover, with the highest change in aBMD defined as being in the upper quintile of annual % bone loss, high bone turnover as being in the upper quintile of bone turnover and lower bone turnover as

being in the lower quintiles of bone turnover, with the results expressed as odds ratios (OR) and 95% CI. Adjustments were made for age, centre, BMI, lifestyle and endocrine factors.

We also determined the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of bone turnover markers. The sensitivity is the probability on high bone turnover among men in the upper quintile of annual % bone loss and the specificity is the probability on lower bone turnover among men in the lower quintiles of annual % bone loss. The PPV reports the probability on the upper quintile of annual % bone loss among men with high bone turnover and the NPV the probability on the lower quintiles of annual % bone loss among men with lower bone turnover. Statistical analysis was performed using STATA version 9.2 (http://www.stata.com).

Results

Subjects

From a total of 847 subjects (451 in Leuven, 396 in Manchester) who participated in the baseline study, 716 subjects (84.5%) participated in the follow-up study (388 in Leuven, 328 in Manchester). 33 subjects (3.9%) died following the baseline survey and 98 (11.6%) were lost to follow-up. Of the 716 subjects who took part in both the baseline and follow-up surveys, 194 men were excluded because of missing baseline bone turnover markers or paired hip/spine aBMD measurements. 34 subjects taking bone active therapies (calcium, vitamin D, bisphosphonates, glucocorticoids) and 1 subject who lost more than 43% of his body weight after gastric bypass surgery were also excluded, leaving 487 subjects, of whom 243 were older than 60 years. No significant differences were observed between the 487 subjects included in the analysis and the 229 subjects excluded. Both groups were similar in terms of baseline age, height, weight, BMI, physical activity, smoking, alcohol consumption, bone turnover markers and aBMD at the hip and lumbar spine.

Mean time between the baseline and follow-up surveys was 4.3 years (range 2.7-6.3). More than 99% of subjects had their repeat DXA scan after 3.0 years or more, while no one had the follow-up DXA scan within 2.7 years after the baseline scan. At baseline, the mean age of participants was 60.1 (SD=10.4) years and mean BMI 27.1 kg/m^2 (SD=3.5). Details of the subject characteristics at baseline are shown in table 1.

aBMD and change in aBMD between baseline and follow-up

Mean aBMD at the femoral neck, total hip and lumbar spine was 810.1 (SD=124.5), 1020.1 (SD=141.6) and 1053.8 (SD=174.4) mg/cm² respectively at baseline, and 798.5 (SD=125.7), 1010.1 (SD=142.0) and 1068.0 (SD=181.2) mg/cm² respectively at follow-up (Table 2). Over a mean follow-up of 4.3 years, men had a significant loss of 11.641 (SD=35.81) mg/cm² (-1.39%; SD=4.40) at the femoral neck and 9.993 (SD=33.79) mg/cm² (-0.95%; SD=3.33) at the total hip. The loss of aBMD at the femoral neck and total hip increased non-significantly with increasing age, from -9.392 mg/cm² in men aged 40-49 years to -17.038 mg/cm² in men aged 70-79 years at the femoral neck (p^{trend}=0.108) and from -8.719 mg/cm² in men aged 40-49 years to -12.653 mg/cm² in men aged 70-79 years at the total hip (p^{trend}=0.361). A significant gain in aBMD of 14.178 (SD=43.64) mg/cm² (1.38%; SD=4.18) was observed at the lumbar spine. This gain in aBMD increased significantly across the age groups, from 0.326 mg/cm² in men aged 40-49 years to 18.372 mg/cm² in men aged 70-79 years (p^{trend}=0.001). Per year, men lost on average 0.32 (SD=1.04) % aBMD at the femoral neck and 0.22 (SD=0.77) % at the total hip and gained on average 0.32 (SD=0.97) % at the spine.

Bone turnover markers and change in aBMD

Each SD increase in β-CTX was associated with a small but statistically significant greater annual % decrease in aBMD at the femoral neck and total hip (0.143% and 0.142% per year

respectively) (Table 3). These associations remained significant after adjustment for age, centre and BMI. Loss of aBMD at the femoral neck and total hip increased with increasing quintile of β-CTX (p^{trend}=0.023 and 0.002 respectively), though only men with β-CTX in the highest quintile had a significantly larger bone loss compared to those with β-CTX in the lowest quintile (Fig. 1). Per SD increase in β-CTX, there also was an annual 0.152% less increase in lumbar spine aBMD, and the result remained significant after adjustment for age, centre and BMI (Table 3). The gain in lumbar spine aBMD decreased with increasing quintile of β-CTX (p^{trend}=0.001), but only men with β-CTX in the fourth and the fifth quintile had significantly less gain in aBMD compared to those with β-CTX in the lowest quintile (Fig. 1).

Similarly, each SD increase in PINP was associated with a 0.102% and 0.096% per year greater loss in aBMD at the femoral neck and total hip respectively and a 0.132% per year less gain in lumbar spine aBMD. These annual % changes in aBMD were small, but statistically significant. Also after adjustment for age, centre and BMI, each increase in PINP was associated with greater bone loss at the femoral neck and total hip, and less marked gain in lumbar spine aBMD (Table 3). The loss of femoral neck and total hip aBMD increased non-significantly across the quintiles of PINP (p^{trend}=0.241 and p^{trend}=0.124 respectively), while the gain in lumbar spine aBMD significantly decreased across the quintiles of PINP (p^{trend}=0.018) (Fig. 1).

After stratification by age (40-59 years *vs* 60-79 years), the association between bone turnover markers and change in aBMD was not significantly different in both age-groups at the lumbar spine, while at the femoral neck and total hip, the associations were significant in the older age-group, but not in the younger (Table 3).

The age, centre and BMI-adjusted associations between bone turnover markers and change in aBMD remained significant after further adjustment for PASE, lifestyle (smoking and alcohol consumption) and serum hormone concentrations of total T, total E_2, SHBG, PTH and

IGF1. The results were the same when adjusting for free or bioavailable T instead of total T and for free or bioavailable E_2 instead of total E_2. The results were also similar with absolute change in aBMD as the outcome instead of annual % change in aBMD, both unadjusted as well as adjusted for age, centre, BMI and further adjusted for baseline aBMD (data not shown).

Prediction of bone loss

Each SD increase in the baseline level of β-CTX or PINP was associated with a 31% to 96% increased risk to have a femoral neck and/or total hip aBMD in a lower quintile at follow-up as compared to baseline (Supplementary table).

Furthermore, after categorizing β-CTX and PINP in quintiles with the 4 lower quintiles as the referent and the outcome defined as the upper quintile of annual % bone loss, men aged 60-79 years with β-CTX in the upper quintile were 4.27 (95% CI 2.09-8.73) and 3.73 (95% CI 1.84-7.57) times more likely to be in the upper quintile of annual % bone loss at the femoral neck and total hip respectively compared to those with β-CTX in the lower quintiles (Table 4). For PINP, these ORs were 2.22 (95% CI 1.10-4.47) and 2.50 (95% CI 1.26-4.96) respectively. With the first quintile of β-CTX and PINP as the referent, only men with β-CTX and PINP in the highest quintile consistently were more likely to be in the upper quintile of annual % bone loss at the femoral neck and total hip, but the test for trend across the quintiles of β-CTX and PINP was significant (Table 4). The ORs remained significant after adjustment for age, centre, BMI and further adjustment for PASE, lifestyle and hormones.

Table 5 shows sensitivity, specificity, PPV and NPV of β-CTX and PINP. The sensitivity of β-CTX and PINP were 35.8% and 30.2% respectively at the femoral neck and 33.3% and 31.6% respectively at the total hip. Consequently, the PPV of β-CTX and PINP were low, respectively 46.3% and 34.0% at the femoral neck and 46.3% and 38.3% at the total hip.

Discussion

In this prospective study of middle-aged and elderly European men, the mean aBMD decreased by 0.32% per year at the femoral neck and by 0.22% per year at the total hip, and increased by 0.32% per year at the lumbar spine. Higher baseline levels of bone turnover markers β-CTX and PINP were significantly associated with higher loss of aBMD at the femoral neck and total hip in the whole cohort as well as in those aged 60-79 years, but not in those aged 40-59 years. These associations were independent of age, centre and BMI. In the older men, high bone turnover was predictive of high bone loss at both hip sites, though the sensitivity was low and bone turnover markers are therefore unlikely to be of clinical utility in predicting accelerated hip bone loss at the individual level.

Loss of aBMD at the femoral neck and total hip was observed in the youngest age-group and increased across the age groups, although this age-related trend was not significant. Bone loss starts in early adulthood because of a negative balance in the BMU, by which less bone is replaced than resorbed in each BMU. This loss is however small since the remodeling rate in these young adults is low [29,30]. With ageing, the same or a higher rate of remodeling removing bone from this constantly reducing bone mass is responsible for an acceleration of bone loss. In middle-aged men, there is no increase in remodeling as seen in postmenopausal women, and bone loss proceeds as the result of reduced bone formation in each BMU. In elderly men, bone remodeling may increase due to secondary hyperparathyroidism [29,31,32]. In our cohort, the mean % bone loss over 4.3 years of follow-up was small, but exceeded the Leuven precision errors of DXA at the femoral neck and total hip, and was comparable to the mean % bone loss in other male cohorts of similar age [6,8,33,34]. The gain in lumbar spine aBMD, which significantly increased across the age groups, probably reflects the age-related increased incidence of lumbar spondylosis and aortic calcification and is consistent with data from other prospective studies [6,8,11].

Higher levels of β-CTX and PINP were significantly associated with a small, but statistically significant greater loss of aBMD at both the femoral neck and total hip. Similar associations were observed in MrOS [7]. In MINOS, however, this association was only observed at the total hip, and also other male cohorts failed to show an association between bone turnover markers and bone loss at the femoral neck [6,8-11,14]. It has been suggested that age-related development of osteophytes in the femoral neck and pathological calcifications in the fibrous tissues around the femoral neck may be one potential explanation for the weaker correlation between bone turnover markers and bone loss at the femoral neck as compared to the total hip [6,35]. We did, however, not observe such a difference between the two hip sites.

After dividing the whole cohort in a younger (<60 years) and an older subgroup (≥60 years), the association between markers of bone turnover and bone loss at the femoral neck and total hip was only significant in the older subgroup. This is in accordance with a cross-sectional study in men in which the association between bone turnover markers and aBMD was not significant before the age of 60 years and moderately negative after the age of 60 years [36]. This may reflect the aforementioned observation that bone remodeling does not accelerate in middle-aged men, contrary to postmenopausal women, and increases in the elderly [29,31,32]. Also the relatively short time of follow-up may contribute to the lack of the association between bone turnover and bone loss in the younger men. This is the first prospective study that has assessed the relationship between bone turnover and hip bone loss in middle-aged and older men separately.

The association between higher baseline levels of bone turnover markers and change in aBMD remained significant after adjusting for physical activity, smoking and alcohol consumption as well as for serum concentrations of total T, total E_2, SHBG, PTH and IGF1. The results were the same when adjusting for free or bioavailable T instead of total T and for free or bioavailable E_2 instead of total E_2. Thus, we were able to confirm in a longitudinal

setting the result of our previous cross-sectional report of EMAS, which showed that, after adjustment for E_2, T, SHBG, PTH and IGF1, β-CTX and PINP remained negatively associated with aBMD at the total hip and lumbar spine [15]. Endocrine factors that are known to regulate bone loss may do so by influencing bone turnover. In men, as in women, there is indeed a well-established relationship between hormone levels and bone remodeling rate, with a negative association between sex hormones, especially E_2, and markers of bone turnover [15,37] and a positive association with SHBG, PTH and IGF1 [15]. In this study, the association between baseline markers of bone turnover and bone loss was not markedly affected by adjustment for hormones, which may reflects the observation that most men in EMAS were relatively healthy men with sex hormones within the normal range. Indeed, depending on the cut-off [38], the prevalence of hypogonadism in this study was 1.9%, 10.3% and 13.0% when defined as total T at < 8 nmol/l, total T at < 11 nmol/l or free T at < 220 pmol/l respectively (data not shown). Men with low baseline levels of T tended to have higher baseline levels of β-CTX and PINP, and there was also a trend towards men with low T having greater loss of aBMD though this did not attain statistical significance (data not shown). Further work is required to determine the influence of sex hormones on the relationship between bone turnover and subsequent bone loss.

Conflicting results about the association between bone turnover markers and change in aBMD between studies may be explained by preanalytical and analytical variability in the measurement of bone turnover markers [4,5]. Non-modifiable determinants of preanalytical variability include age, gender and menopausal status. Bone turnover markers indeed increase with age and at the menopause, and are higher in older women than in men. Fasting status is important for some markers, with for example a 20% decrease in the level of β-CTX after breakfast [4], and especially markers of bone resorption have a strong circadian pattern with the lowest values in the afternoon and evening. Moreover, the measurement of some urinary

bone turnover markers may be inaccurate due to an incomplete urine collection, renal impairment or muscle wasting [6]. Analytical variability of bone turnover markers, expressed by the CV of the measurement, depends on the specific marker and the measurement method. To avoid some of the modifiable sources of variability, we have measured serum β-CTX and PINP, the markers that have been proposed by the International Osteoporosis Foundation (IOF) as the reference marker for bone resorption and bone formation respectively, on a fasting morning blood sample using previously published measurement methods [4,21,22].

However, despite the use of reference markers of bone turnover and validated measurement methods, still other issues limit the routine use of bone turnover markers in predicting bone loss in clinical practice. Indeed, other determinants of preanalytical variability that influence the level of bone turnover markers in individual patients have to be taken into account as well. For example, bone turnover markers decrease in patients on drugs such as glucocorticoids and antiresorptives, while bone turnover markers increase in patients with hyperthyroidism, primary or secondary hyperparathyroidism and chronic kidney or liver disease. Bone turnover markers also increase within a few weeks after a fracture, and markers of bone formation decrease and markers of bone resorption increase during prolonged periods of immobility. Moreover, as we showed in this study, the PPV of β-CTX and PINP are low, implying that bone turnover markers have limited ability to identify in individual subjects the small subgroup of fast bone losers among the large group of men with relatively stable aBMD. Men aged 60-79 years with β-CTX or PINP in the upper quintile were 2.22 to 4.27 times more likely to be in the upper quintile of annual % hip bone loss, compared to those with the lower quintiles of these markers. A decrease in the quintile of femoral neck or total hip aBMD between baseline and follow-up was also predicted by higher baseline levels of β-CTX and PINP. However, higher odds ratios are needed for a risk factor to be useful in predicting a condition with low prevalence. Therefore, the PPV of the upper quintile of β-

CTX and PINP to detect men in the upper quintile of annual hip BMD loss was still only 34.0% to 46.3%. Consequently, one should not decide whether or not to initiate osteoporosis treatment based on the baseline level of bone turnover markers.

Our study used standard methods with repeat measurements over a 4.3 year follow-up. There are however some limitations which need to be considered. First, the mortality-adjusted follow-up rate in our cohort was 84.5%. Using data from the baseline survey, compared to those who took part in the follow-up phase, those who were lost to follow-up were not different with respect to age and frequency of alcohol consumption, but they were more likely to be current smokers and to have a slightly higher PASE score [17]. This is, however, unlikely to have influenced the results, which were based on an internal comparison of responders. Secondly, our data were based on a predominantly Caucasian group of middle-aged and elderly men and the results should be extrapolated beyond this with caution. Thirdly, our analysis was based on an assessment of bone turnover markers at one time point and cannot therefore exclude any effect of change in bone turnover on bone mass during the interval period for which repeat measurement of bone turnover markers would be required. Finally, follow-up of the largely middle-aged overall cohort in EMAS is still too short to study fracture endpoints.

In summary, over a mean follow-up of 4.3 years, middle-aged and elderly European men had a yearly loss of aBMD of 0.32% at the femoral neck and 0.22% at the total hip. In the whole cohort as well as in the older subset separately higher baseline levels of β-CTX and PINP were associated with loss of aBMD at the femoral neck and total hip. Older men with high bone turnover have a higher risk of accelerated hip bone loss, but the PPV is low and bone turnover markers are therefore unlikely to be of clinical utility in predicting accelerated bone loss in individual men.

Disclosure

Evelien Gielen, Terence O'Neill, Stephen Pye, Judith Adams, Kate Ward, Frederick Wu, Michaël Laurent, Frank Claessens, Steven Boonen, Dirk Vanderschueren and Sabine Verschueren declare that they have no conflict of interest

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Table 1. Subject characteristics at baseline

Variable (*N*=487)	Mean (SD)
Age (years)	60.1 (10.4)
Weight (kg)	83.0 (12.4)
Height (cm)	174.8 (6.9)
BMI (kg/m^2)	27.1 (3.5)
PASE score (0-1100)	208.6 (80.8)
β-CTX (pg/ml)	326.6 (155.5)
PINP (ng/ml)	42.0 (19.4)
Total T (nmol/l)	18.1 (6.2)
Free T (pmol/l)	319.4 (88.6)
Bioavailable T (nmol/l)	8.0 (2.3)
Total E_2 (pmol/l)	76.1 (24.0)
Free E_2 (pmol/l)	1.3 (0.4)
Bioavailable E_2 (pmol/l)	53.4 (16.7)
SHBG (nmol/l)	42.7 (19.5)
PTH (pg/ml)	28.7 (10.9)
IGF1 (ng/ml)	137.0 (40.6)
	%
Ever smoked	
no	40.6
yes	58.8
don't remember	0.6
Alcohol consumption	
none or < once a week	21.2
> once a week	78.8

Results expressed as mean (SD) and percentage. SD, standard deviation; β-CTX, β C-terminal cross-linked telopeptide of type I collagen; PINP, N-terminal propeptide of type I procollagen; T, testosterone; E_2, estradiol; SHBG, sex hormone-binding globulin; PTH, parathyroid hormone; IGF1, insulin-like growth factor-1

Table 2. Baseline and follow-up aBMD, absolute change [a] and % change per year [b]

	N	Baseline	Follow-up	Absolute change	% change/year
aBMD		mg/cm²	mg/cm²	mg/cm²	% / year
Femoral neck					
40-49	104	861.0 (127.1)	851.6 (130.3)	-9.392 (37.67)	-0.25 (0.95)
50-59	140	811.4 (129.3)	801.8 (130.5)	-9.593 (33.86)	-0.27 (1.01)
60-69	133	776.3 (108.6)	765.2 (107.7)	-11.093 (35.85)	-0.30 (1.11)
70-79	110	801.2 (119.3)	784.2 (119.9)	-17.038 (36.32)	-0.49 (1.08)
40-79	487	810.1 (124.5)	798.5 (125.7)	-11.641 (35.81)	-0.32 (1.04)
Total hip					
40-49	104	1062.0 (145.7)	1053.2 (143.9)	-8.719 (37.28)	-0.19 (0.74)
50-59	140	1012.6 (148.6)	1003.8 (146.7)	-8.797 (30.47)	-0.18 (0.71)
60-69	133	993.5 (124.1)	983.5 (123.5)	-10.047 (32.75)	-0.21 (0.81)
70-79	110	1022.0 (141.1)	1009.4 (147.2)	-12.653 (35.84)	-0.29 (0.83)
40-79	487	1020.1 (141.6)	1010.1 (142.0)	-9.993 (33.79)	-0.22 (0.77)
Lumbar spine					
40-49	104	1057.6 (150.4)	1058.0 (154.7)	0.326 (31.98)	0.03 (0.69)
50-59	140	1033.3 (159.5)	1046.5 (165.1)	13.217 (39.32)	0.29 (0.93)
60-69	133	1027.9 (164.2)	1050.4 (174.9)	22.551 (47.69)	0.52 (1.09)
70-79	110	1107.8 (211.6)	1126.1 (218.0)	18.372 (50.14)	0.40 (1.05)
40-79	487	1053.8 (174.4)	1068.0 (181.2)	14.178 (43.64)	0.32 (0.97)

Results expressed as mean (SD)

[a] Absolute change = follow-up – baseline

[b] % change per year = ([follow-up – baseline]/baseline)*100/time between baseline and follow-up

Table 3. Association between annual % change [a] in aBMD and baseline BTMs (per SD)

	Annual % change in femoral neck aBMD	
	unadjusted	adjusted [b]
	β coefficient (95% CI)	
z-β-CTX (per SD)		
overall	**-0.143 (-0.235, -0.050)****	**-0.151 (-0.245, -0.056)****
40-59	-0.000 (-0.133, 0.132)	0.028 (-0.107, 0.163)
60-79	**-0.262 (-0.391, -0.134)*****	**-0.295 (-0.424, -0.166)*****
p^{diff}	**0.005****	**0.007****
z-PINP (per SD)		
overall	**-0.102 (-0.195, -0.009)***	**-0.106 (-0.200, -0.012)***
40-59	0.053 (-0.093, 0.199)	0.070 (-0.080, 0.221)
60-79	**-0.191 (-0.312, -0.070)****	**-0.207 (-0.328, -0.086)****
p^{diff}	**0.013****	**0.018****

	Annual % change in total hip aBMD	
	unadjusted	adjusted [b]
	β coefficient (95% CI)	
z-β-CTX (per SD)		
overall	**-0.142 (-0.210, -0.075)*****	**-0.154 (-0.223, -0.084)*****
40-59	-0.061 (-0.158, 0.036)	-0.062 (-0.163, 0.038)
60-79		**-0.211 (-0.306, -0.117)*****
p^{diff}	**0.030****	**0.032****
z-PINP (per SD)		
overall	**-0.096 (-0.164, -0.028)***	**-0.101 (-0.170, -0.032)****
40-59	-0.002 (-0.109, 0.105)	0.003 (-0.110, 0.116)
60-79	**-0.150 (-0.240, -0.060)****	**-0.157 (-0.247, -0.067)****
p^{diff}	**0.040***	**0.047***

	Annual % change in lumbar spine aBMD	
	unadjusted	adjusted [b]
	β coefficient (95% CI)	
z-β-CTX (per SD)		
overall	**-0.152 (-0.238, -0.066)****	**-0.120 (-0.206, -0.033)****
40-59	**-0.127 (-0.240, -0.015)***	-0.086 (-0.199, 0.028)
60-79	**-0.159 (-0.286, -0.032)***	**-0.142 (-0.271, -0.013)***
p^{diff}	0.718	0.693
z-PINP (per SD)		
overall	**-0.132 (-0.218, -0.046)****	**-0.112 (-0.198, -0.026)***
40-59	-0.060 (-0.186, 0.065)	0.000 (-0.127, 0.128)
60-79	**-0.172 (-0.290, -0.054)****	**-0.162 (-0.280, -0.044)****
p^{diff}	0.216	0.174

aBMD = areal bone mineral density; BTM = bone turnover marker; SD = standard deviation; β coefficient = mean difference in % change/year; CI = confidence interval

***p<0.05, **p<0.01, ***p<0.001**

p^{diff} = p value for difference between 40-59 and 60-79 age groups

[a] % change per year = ([follow-up – baseline] / baseline) * 100/ time between baseline and follow-up

[b] adjusted for age, centre, BMI